

**AMENDMENTS TO THE CLAIMS**

**This listing of claims will replace all prior versions and listings of claims in the application:**

**LISTING OF CLAIMS:**

Claim 1. (currently Amended): A diagnostic or prognostic assay for ~~a cancer~~ prostate cancer or liver cancer in a subject, said cancer characterized by abnormal methylation of cytosine at at least one CpG site in a target region within the glutathione S-transferase (GST) Pi gene and/or its regulatory flanking sequences, wherein said assay comprises the steps of:

- (i) isolating DNA from a test subject,
- (ii) carrying out amplification of said isolated DNA so as to amplify a target region of the GST-Pi gene and/or its regulatory flanking sequences which includes a site or sites at which abnormal cytosine methylation characteristic of the cancer occurs, the amplification being selective in that it only amplifies the target region if the said site or sites at which abnormal cytosine methylation occurs is/are methylated, and
- (iii) detecting the presence of amplified DNA, wherein the detection of amplified DNA is indicative of methylation, and thereby indicative of said cancer,

wherein the amplifying step (ii) is used to amplify a target region, wherein said target region is within the GST-Pi gene and/or its regulatory flanking sequences ~~and comprises CpG site -43 represented by nucleotides 442-343 of SEQ ID NO:52 to CpG site +55 represented by nucleotides \_\_\_ and \_\_\_ of SEQ ID NO:54 defined by (and inclusive of) sites 342-343 of SEQ ID NO: 52 to CpG sites 581-582 of SEQ ID NO: 54,~~

wherein the isolated DNA is not treated with a methylation sensitive restriction endonuclease prior to amplification in step (i).

Claim 2. (original): An assay according to Claim 1, wherein prior to the amplifying step, the isolated DNA is treated such that unmethylated cytosines are converted to uracil or another nucleotide capable of forming a base pair with adenine while methylated cytosines are unchanged or converted to a nucleotide capable of forming a base pair with guanine.

Claim 3. (original): An assay according to Claim 1, wherein the amplifying step involves polymerase chain reaction (PCR) amplification.

Claim 4. (previously amended): An assay according to Claim 2, wherein said amplification step comprises PCR amplification utilizing a reverse primer including guanine at at least one site whereby, upon the reverse primer annealing to the treated DNA, said guanine will either form a base pair with a methylated cytosine (or another nucleotide to which the methylated cytosine has been converted through said treatment) if present, or will form a mismatch with uracil (or another nucleotide to which unmethylated cytosine has been converted through said treatment).

Claim 5. (previously amended): An assay according to claim 4, wherein said PCR amplification utilizes a forward primer including cytosine at least one site(s) corresponding to cytosine nucleotides that are abnormally methylated in the DNA of a subject with the cancer being assayed.

Claim 6. (original): An assay according to Claim 5, wherein the primers are of 12 to 30 nucleotides in length.

Claim 7. (previously amended): An assay according to claim 6, wherein the primers are selected so as to anneal to a sequence within the target region that includes two to four cytosine nucleotides that are abnormally methylated in the isolated DNA of a subject with the cancer being assayed.

Claim 8. (original): An assay according to claim 2, wherein the treatment of the isolated DNA involves reacting the isolated DNA with bisulphite.

Claim 9. (original): An assay according to claim 8, wherein the amplifying step involves polymerase chain reaction (PCR) amplification.

Claim 10. (original): An assay according to claim 9, wherein said PCR amplification utilizes a reverse primer including guanine at at least one site whereby, upon the reverse primer annealing to the treated DNA, said guanine will either form a base pair with a methylated cytosine if present, or will form a mismatch with uracil.

Claim 11. (previously amended): An assay according to claim 10, wherein said PCR amplification utilizes a forward primer including cytosine at at least one site(s) corresponding to cytosine nucleotides that are abnormally methylated in the isolated DNA of a subject with the cancer being assayed.

Claim 12. (original): An assay according to claim 11, wherein the primers are of 12 to 30 nucleotides in length.

Claim 13. (previously amended): An assay according to claim 12, wherein the primers are selected so as to anneal to a sequence within the target region that includes two to four cytosine nucleotides that are abnormally methylated in the DNA of a subject with cancer being assayed.

Claim 14. (previously amended): An assay according to Claim 1, wherein said DNA is isolated from cells from tissue, blood, blood serum, blood plasma, semen, urine, lymph, or bone marrow.

Claim 15. (cancelled)

Claim 16. (cancelled)

Claim 17. (previously amended): An assay according to claim 16, wherein the cancer to be assayed is prostate cancer.

Claim 18 (original): An assay according to claim 17, wherein the amplifying step is used to amplify a target region within the region of the GST-Pi gene and its regulatory flanking sequences defined by (and inclusive of) CpG sites -43 to +53.

Claim 19. (original): An assay according to claim 17, wherein the amplifying step is used to amplify a target region within the region of the GST-Pi gene and its regulatory flanking sequences defined by (and inclusive of) CpG sites -43 to +10.

Claim 20. (original): An assay according to claim 17, wherein the amplifying step is used to amplify a target region within the region of the GST-Pi gene and its regulatory flanking sequences defined by (and inclusive of) CpG sites -43 to -14.

Claim 21. (original): An assay according to claim 17, wherein the amplifying step is used to amplify a target region within the region of the GST-Pi gene and its regulatory flanking sequences defined by (and inclusive of) CpG sites -43 to -8.

Claim 22. (original): An assay according to claim 17, wherein the amplifying step is used to amplify a target region within the region of the GST-Pi gene and its regulatory flanking sequences defined by (and inclusive of) CpG sites -43 to -8.

Claim 23. (previously amended): An assay according to claim 1, wherein if either or both of the reverse or forward primers anneal to a sequence within the target region that includes any or all of CpG sites -36, -32, -23, -20, -19 and -14, then said PCR amplification further utilizes equivalent reverse and/or forward primers including a redundant nucleotide(s) at the position(s) within their sequence(s) corresponding to cytosine or methylated cytosine of CpG sites -36, -32, -23, -20, -19 and -14.

Claim 24. (original): An assay according to claim 17, wherein the amplifying step is used to amplify a target region within the region of the GST-Pi gene and its regulatory flanking sequences defined by (and inclusive of) CpG sites +9 to +53.

Claim 25. (previously amended): An assay according to claim 17, wherein the amplification step is used to amplify a target region within the region of the GST-Pi gene and its regulatory flanking sequences defined by (and inclusive of) CpG sites +1 to +53.

Claim 26.(previously amended): An assay according to claim 17, wherein the amplification involves PCR amplification using primer pairs consisting of a forward and reverse primer selected from each of the following groups:

Forward Primers

CGCGAGGTTTTCGTTGGAGTTTCGTCGTC (SEQ ID NO: 1)

CGTTATTAGTGAGTACGCGCGGTTC (SEQ ID NO: 2)

YGGTTTTAGGGAATTTTTTTTCGC (SEQ ID NO: 3)

YGGYGYGTTAGTTYGTTGYGTATATTTC (SEQ ID NO: 4)

GGAATTTTTTTTCGCGATGTTYGGCGC (SEQ ID NO: 5)

TTTTTAGGGGGTTYGGAGCGTTTC (SEQ ID NO: 6)

GGTAGGTTGYGTTTATCGC (SEQ ID NO: 7)

Reverse Primers

TCCCATCCCTCCCCGAAACGCTCCG (SEQ ID NO: 8)

GAAACGCTCCGAACCCCCTAAAAACGCTAACG (SEQ ID NO: 9)

CRCCCTAAAATCCCCRAAATCRCCGCG (SEQ ID NO: 10)

ACCCCRACRACCRCTACACCCRAACGTCG (SEQ ID NO: 11)

CTCTTCTAAAAAATCCCRCAACTCCCGCCG (SEQ ID NO: 12)

AAAACRCCCTAAAATCCCCGAAATCGCCG (SEQ ID NO: 13)

AACTCCCRCCGACCCCAACCCCGACGACCG (SEQ ID NO: 14)

AAAAATTCRAATCTCTCCGAATAAACG (SEQ ID NO: 15)

AAAAACCRAAATAAAAACCACACGACG (SEQ ID NO: 16),

wherein Y is C, T or a mixture thereof, and R is A, G or a mixture thereof.

Claim 27. (previously amended): An assay according to claim 17, wherein the amplification step involves PCR amplification using primer pairs consisting of a forward and reverse primer selected from each of the following groups:

Forward Primers

CGCGAGGTTTTCTGTTGGAGTTTCGTCGTC (SEQ ID NO: 1)

CGTTATTAGTGAGTACGCGCGGTTC (SEQ ID NO: 2)

Reverse Primers

GAAACGCTCCGAACCCCCTAAAAACCGCTAACG (SEQ ID NO: 9).

Claim 28. (previously amended): An assay according to claim 17, wherein the amplification step involves PCR amplification using primer pairs consisting of a forward and reverse primer selected from each of the following groups:

Forward Primers

YGGTTTTAGGGAATTTTTTTTCGC (SEQ ID NO: 3)

YGGYGYGTTAGTTYGTTGYGTATATTC (SEQ ID NO: 4)

GGGAATTTTTTTTCGCGATGTTYGGCGC (SEQ ID NO: 5)

Reverse Primers

CRCCCTAAAATCCCCRAAATCRCCGCG (SEQ ID NO: 10)

ACCCCRACRACCRCTACACCCRAACGTCG (SEQ ID NO: 11)

CTCTTCTAAAAAATCCCRCAACTCCCGCCG (SEQ ID NO: 12)

AAAACRCCCTAAAATCCCCGAAATCGCCG (SEQ ID NO: 13)

AACTCCCRCCGACCCCAACCCCGACGACCG (SEQ ID NO: 14),

wherein Y is C, T or a mixture thereof and R is A, G or a mixture thereof.

Claim 29. (previously amended): An assay according to claim 17, wherein the amplification step involves PCR amplification using primer pairs consisting of a forward and reverse primer selected from each of the following groups:

Forward Primers

TTTTTAGGGGGTTYGGAGCGTTTC (SEQ ID NO: 6)

GGTAGGTTGYGTTTATCGC (SEQ ID NO: 7)

Reverse Primers

AAAAATTCRAATCTCTCCGAATAAACG (SEQ ID NO: 15)

AAAAACCRAAATAAAAACCACACGACG (SEQ ID NO: 16),

wherein Y is C, T or a mixture thereof, and R is A, G or a mixture thereof.

Claim 30. (previously amended): An assay according to claim 16, wherein the cancer to be assayed is liver cancer.

Claim 31. (previously amended): An assay according to claim 30, wherein the amplification step is used to amplify a target region within the region of the GST-Pi gene and its regulatory flanking sequences defined by (and inclusive of) CpG sites -43 to -14.

Claim 32. (original): An assay according to claim 31, wherein the target region excludes any or all of the CpG sites -36, -32, -23, -20, -19, and -14.

Claim 33. (original): An assay according to claim 30, wherein if either or both of the reverse or forward primers anneal to a sequence within the target region that includes any or all of CpG sites -36, -32, -23, -20, -19 and -14, then said PCR amplification further utilizes equivalent reverse and/or forward primers including a redundant nucleotide(s) at the position(s)

within their sequence(s) corresponding to cytosine or methylated cytosine of CpG sites -36, -32, -23, -20, -19 and -14.

Claim 34. (previously amended): An assay according to claim 30, wherein the amplification step is used to amplify a target region within the region of the GST-Pi gene and its regulatory flanking sequences defined by (and inclusive of) CpG sites +9 to +53.

Claim 35. (cancelled)

Claim 36. (original): An assay according to claim 35, wherein the region of the GST-Pi gene and its regulatory flanking sequences within which the presence of methylated cytosine(s) at a site or sites is determined is selected from the regions defined by (and inclusive of) CpG sites -43 to +53, -43 to +10, -43 to -14, +9 to +53 and +1 to +53.

Claim 37. (previously amended): An assay according to claim 35, wherein the said region of the GST-Pi gene and its regulatory flanking sequences excludes any or all of the CpG sites -36, -32, -23, -20, -19 and -14.

Claim 38. (original): An assay according to claim 36, wherein the region of the GST-Pi gene and its regulatory flanking sequences within which the presence of methylated cytosine(s) at a site or sites is determined is the region defined by (and inclusive of) CpG sites +9 to +53.

Claim 39. (original): An assay according to claim 36, wherein the region of the GST-Pi gene and its regulatory flanking sequences within which the presence of methylated cytosine(s) at a site or sites is determined is the region defined by (and inclusive of) CpG sites +1 to +53.

Claim 40. (original): An assay according to claim 35, wherein prior to the determination step, the isolated DNA is treated such that unmethylated cytosines are converted to uracil or another nucleotide capable of forming a base pair with adenine while methylated cytosines are unchanged or are converted to a nucleotide capable of forming a base pair with guanine.



Claim 41. (original): An assay according to claim 40, wherein the treatment of the isolated DNA involves reacting the isolated DNA with bisulphite.

Claim 42. (original): An assay according to claim 35, wherein the determination step involves selective hybridisation of oligonucleotide/polynucleotide/peptide-nucleic acid (PNA) probe(s).

Claim 43. (original): An assay according to claim 42, wherein if the probe(s) hybridise to a sequence within the target region that includes any or all of CpG sites -36, -32, -23, -20, -19 and -14, then said selective hybridisations further utilizes equivalent probe(s) including a redundant nucleotide(s) at the position(s) within their sequence(s) corresponding to cytosine or methylated cytosine of CpG sites -36, -32, -23, -20, -19 and -14.

Claim 44. (cancelled)

Claim 45. (cancelled)

Claims 46.-48. (cancelled)

Claim 49 (withdrawn): A primer or probe comprising a nucleotide sequence selected from the group consisting of:

CGCGAGGTTTTCGTTGGAGTTTCGTCGTC (SEQ ID NO: 1)

CGTTATTAGTGAGTACGCGCGGTTC (SEQ ID NO: 2)

YGGTTTTAGGGAATTTTTTTTCGC (SEQ ID NO: 3)

YGGYGYGTTAGTTYGTTGYGTATATTC (SEQ ID NO: 4)

GGGAATTTTTTTTCGCGATGTTYGGCGC (SEQ ID NO: 5)

TTTTTAGGGGGTTYGGAGCGTTTC (SEQ ID NO: 6)

GGTAGGTTGYGTTTATCGC (SEQ ID NO: 7)

AAAAATTCRAATCTCTCCGAATAAACG (SEQ ID NO: 8)

AAAAACCRAAATAAAAACCACACGACG (SEQ ID NO: 9)

TCCCATCCCTCCCCGAAACGCTCCG (SEQ ID NO: 10)

GAAACGCTCCGAACCCCCTAAAAACGCTAACG (SEQ ID NO: 11)

CRCCCTAAAATCCCCRAAATCRCCGCG (SEQ ID NO: 12)

ACCCCRACRACRCTACACCCRAACGTCG (SEQ ID NO: 13)

CTCTTCTAAAAAATCCCRCAACTCCCGCCG (SEQ ID NO: 14)

AAAACRCCCTAAAATCCCCGAAATCGCCG (SEQ ID NO: 15)

AACTCCCRCCGACCCCAACCCCGACGACCG, (SEQ ID NO: 16),

wherein Y is a mixture of C and T, and R is a mixture of A and G.

Claim 50. (withdrawn): A probe comprising a nucleotide sequence selected from the group consisting of:

AAACCTAAAAAATAAACAAACAA (SEQ ID NO: 17)

GGGCCTAGGGAGTAAACAGACAG (SEQ ID NO: 18)

CCTTTCCCTCTTTCCCARRTCCCCA (SEQ ID NO: 19)

TTTGGTATTTTTTTTCGGGTTTTAG (SEQ ID NO: 20)

CTTGGCATCCTCCCCCGGGCTCCAG (SEQ ID NO: 21)

GGYAGGGAAGGGAGGYAGGGGYTGGG (SEQ ID NO: 22)

Claims 51-76. (cancelled)